IN-VITRO ANTIBACTERIAL ACTIVITY OF *PSIDIUM GUAJAVA L.* LEAF EXTRACTS AGAINST CLINICALLY IMPORTANT PATHOGENS *Staphylococcus aureus* AND *Escherichia coli.*

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ABSTRACT

Today, around 80% of the world's population uses plants that have been used for thousands of years to cure a variety of diseases due to their effectiveness, availability, and lack of adverse effects. The crude extract of the *psidium guajava* leaves exhibited antibacterial and antifungal properties. In this work, *p.guajava linn* leaf extract was tested for its ability to combat both gram-positive and gram-negative bacteria. Guava plants that have been used for the treatment of various diseases for thousands of years due to their effectiveness, availability, and lack of side effects. These plants are used to link the activity of bacteriostatic and fungistatic strains using the turbidity method and to link that activity to the plants components. *P.guajava* water extract is has antibacterial action against *Escherichia coli and staphylococcus aureus*. It indicates via Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC), and Antibacterial Sensitivity (ABST) techniques. Guava leaf extract is an important source of antibacterial agent since it is effective against *Staphylococcus aureus* and *Escherichia coli.*

KEYWORDS:

Psidium guajava, antibacterial activity, *Staphylococcus aureus* and *Escherichia coli.*

INTRODUCTION

Psidium guajava, often known as guava, goyave, or goyavier, is a member of the Myrtaceae family. The herb is traditionally used as a medicine and to treat a variety of human illnesses. It has been a component of a variety of dishes and desserts. Different plant parts are employed in folk medicine to treat conditions like ulcers, lesions, wounds, diarrhoea, cholera, hypertension, obesity, and diabetes mellitus. It has a high concentration of ascorbic acid and is a great source of antioxidants. Saponins, tannins, flavonoids, and alkaloids are the main active components. Their effectiveness when used as twigs is due to these compounds. There are numerous chemical components in *P. guajava*.

Its antibacterial, antidiarrheal, antimycobacterial, antihyperglycemic, antimalarial, cytotoxic, and antioxidant effects have been described. Bacterial activity and the methods used to combat different bacteria Plants have long been believed to have medicinal properties. Since prehistory, people have used poultices made from hundreds or perhaps thousands of native plants and consumed infusions of them. In India, various portions of medicinal plants have been utilised for centuries to treat various illnesses. In today's society, examining medications made from plant sources is a hot topic. It is widely acknowledged that eating more fruits and vegetables reduces the risk of developing cancer and heart disease. The idea that fruits and vegetables have bioactive substances with protective properties is appealing.

The young leaves are used as medicinal in India. The leaves are utilised as a hemostatic and anti-inflammatory in China. Cholera, diarrhoea, and vomiting decrease are all treated with the leaf decoction. The leaves are a common therapy option for digestive issues in Brazil. This practise was initially derived from Mexican Aztec medicine. Tannins, flavonoids, essential oils, triterpenoids, and beta-sitosterol are among the chemical substances found. It contains substantial amounts of fatty acid pectins. Using the invitro agar well diffusion method, the inhibitory effects of aqueous and alcoholic extracts of P. guajava (root and leaves) on the

development of Staphylococcus aureus and Escherichia coli, the culprits of intestinal infections in people, were investigated.

The findings supported the use of *P.guajava* in traditional remedies for digestive illnesses caused by bacteria guava leaf or bark extracts have been linked to therapeutic mechanisms against cancer bacterial infection inflammation and pain in animal models of these diseases it is a tiny tree with several sections exhibiting anti-inflammatory, anti-microbial hepatoprotective and anti-diabetic characteristics the main goal of the current study is to determine plants that can be used as herbal alternatives to synthetic medications to treat dental infections our research on the effectiveness of antibacterial action targets *Escherichia coli* and *Staphylococcus aureus* as the targets of our research.

Man has a tendency to overlook the value of herbal medicine despite the fact that plants have long been a true supply of pharmaceuticals research on medicinal plants is advancing and knowledge about these plants is being shared this idea will greatly advance the study of medicinal plants for human benefit and is expected to lessen the need for or dependence on medications while guava fruit paste and cheese are common dishes in florida the west indies and some regions of south america guava plant leaves and bark extract which is prepared by boiling are used in medicinal preparations that are used as treatments for dysentery, diarrhoea and upper respiratory tract infections.

In this study we aim to determine whether the total extracts of *P.guajava* leaves are effective at killing or preventing the growth of foodborne bacteria like *Staphylococcus aureus, Escherichia coli, Salmonella enteritidis* and *Bacillus cereus* which can result in foodborne illness and spoilage we will do this by using various aqueous and organic solvents.

MATERIALS AND METHODS

BACTERIAL STRAINS COLLECTION

Pure culture of *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Microbiology, CMC, Vellore.

IDENTIFICATION OF STRAINS

The organism identified by Gram straining technique.

PLANT LEAF COLLECTION

The fresh leaves of *P. guajava* were collected from Tiruvannamalai in April 2022.

PROCESSING AND EXTRACTION OF PLANT MATERIAL

(1) The leaves were thoroughly washed in running water and then the healthy leaves were separated from these branches.

(2) The leaves were again rinsed in water treated with reverse osmosis and shade-dried over a period of 1-2 weeks at room temperature.

(3) The dried leaves were hand- crushed separately to obtain coarse powder of the leaves.

(4) Subsequently, the fine powder was prepared using a mixer grinder were labeled and stored in the refrigerator at 4°C till further use.

(5) Fresh and dried guava leaves were initially extracted by Methanol, Acetone, Ethyl acetate, Ethanol, Dimethyl sulfoxide (DMSO).

(6) 2 g of fine powder mixed with 10 ml of ethanol mix gentle and intermittent stirring for 15 min every 2 h for 48 h on a rotary shaker.

(7) The crude extract mixture filtered using Whatman filter paper No. 1.

(8) The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary vacuum evaporator, which was kept in a desiccator for further use.

CONCENTRACTION OF EXTRACT

(1) The stock solution of the extract was prepared by dissolving 10 mg of plant extract in 10 ml Methanol.

(2) The following concentration were prepared 20, 15, 10, 5, and 1 µg/ml of the crude extract for determination of antibacterial sensitivity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

(3) A standard anti-bacterial agents, norfloxacin (Belco Pharmaceuticals, Bahadurgarh) served as a positive control.

(4) Dimethylsulfoide (DMSO) was used as a negative control.

MEDIA PREPARATION

(1) Mueller-Hinton agar (MHA) used for antibacterial studies, 10 g of Muller-Hinton agar (MHA) were prepared and dissolving in 50 ml of distilled water.

(2) The media were then sterilized by autoclaving at 15 lb/psi pressure for 15 mins.

(3) After cooling the Muller-Hinton agar (MHA) medium was poured into sterile Petriplates.

ANTIBACTERIAL SENSITIVITY TEST

(1) Cultures were maintained at refrigerated conditions.

(2) For culture studies, the fresh 24-hrs cultures were prepared in the case of *Staphylococcus aureus* and *Escherichia coli.*

(3) The bacterial suspension was prepared in normal saline by transferring the organism from fresh culture (1×10^{8} cells/ml) [Table 1]

(4) To determine the antibacterial activity of the crude extract, 0.2 µl individual bacterial cultures were swabed with a Muller-Hinton agar (MHA) medium in petri plates.

(5) Sterilized filter paper discs (Whatman No. 1;6 mm in diameter) soaked in different beakers containing the dissolved extracts of different concentrations were taken out with sterilized forceps and air-dried and placed on plates with the different organisms.

(6) The plates were incubated at 37°C for 24 hrs at 37°C for *Staphylococcus aureus* and *Escherichia coli* control plate was maintained.

(7) After incubation, the inoculated plates were observed for zones of inhibition in millimeter diameter using a transparent ruler.

(8) The sensitivity or susceptibility of the test bacteria to the standard drug was tested using an inoculated agar plate and norfloxacin served as positive conttol.

(9) The zones of inhibition were measured and compared with those of the plant extract **(Geidam YA *et al.,* 2010).**

(10) The experiment was performed in triplicate, and the results are reported.

MINIMUM INHIBITORY CONCENTRATION (MIC)

(1) The turbidity method or tube dilution method was used for determination of minimum inhibitory concentration (MIC).

(2) The extract was serially diluted to a give a concentration of 20, 15, 10, 5 and 1 µg/ml in test tubes containing 1 ml sterile nutrient broth.

(3) Then, the tubes were inoculated with 100 µl of bacterial suspension and control tube was maintained.

(4) All the tubes were then incubated at 37 °C for 24 hrs and the examined for growth by observing turbidity.

MINIMUM BACTERICIDAL CONCENTRATION (MBC)

(1) The MBC of the plant extract on the bacterial isolates was carried by pipetting out 0.1 ml bacterial culture from the mixture obtained in the determination of MIC tubes which did not show any growth and subculture on to nutrient media and incubated at 37 °C for 24 hrs.

(2) After incubation, the concentration at which there was no single colony of bacteria was taken as MBC. **(Vats M *et al.*, 2009)**

RESULTS

(1) IDENTIFICATION OF MICROORGANISM:

Table 1 identified the organisms of *Staphylococcus aureus* and *Escherichia coli* by gram staining technique (Fig 1 and Fig 2).

(2) ANTI-BACTERIAL SENSITIVITY TEST:

Table 2 shows the result of the anti-bacterial sensitivity testing of the extraction against *Staphylococcus aureus* and *Escherichia coli.* The zone of inhibition recorded the ranged from 12 - 3.0 mm and 10 - 6.0 mm. The result in this work shows that there is variation in the degree of anti-bacterial activities of the extract. The variation in the anti-bacterial activities is presumed to be due to different in the quantity of compounds present in those plant extracts.

(3) MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of the extracts as presented in Table 3 shows that the methanolic extract exhibited antibacterial activity against *Escherichia coli* with minimum inhibitory concentration (MIC) 1µg/ml and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) 20µg/ml. The extract was found to be bacteriostatic in action. The results indicated the presences of different components that might be responsible for antimicrobial activity of the test extraction.

(4) MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Table 4 shows the results of the Minimum bactericidal concentration (MBC) of the plant extract against *Staphylococcus aureus* and *Escherichia coli.* The result showed that the methanolic extract of the individual plants used in the study has a better bactericidal effect(50µg/ml).

This implies that methanolic extract is efficient in inhibiting visible microbial growth and also kill the microbes.

Table 1

Classification and optimal growth parameters for microbes used in this study.

S.No	Bacteria	Classification	Media	Incubation temperature (°C)
1.	*Staphylococcus aureus*	Pathogenic, Gram positive	Nutrient agar	37
2.	*Escherichia coli*	Pathogenic, Gram negative	Nutrient agar	37

Table 2

The result of the antibacterial sensitivity testing of the extract against *Staphylococcus aureus* and *Escherichia coli.*

Concentration (µg/ml)	Zone of inhibition (mm)	
	Escherichia coli	*Staphylococcus aureus*
20	12	10
15	10	9.5
10	9	8
5	7	6
1	3	-

Table 3: MIC and MBC of *P.guajava* methanolic leaf extract against bacterial isolates

Bacterial isolates	***P.guajava* MIC (µg/ml)**	***P.guajava* MBC (µg/ml)**
E.coli	1.0	50
S.aureus	20	50

Fig1. Gram staining of *E. coli*

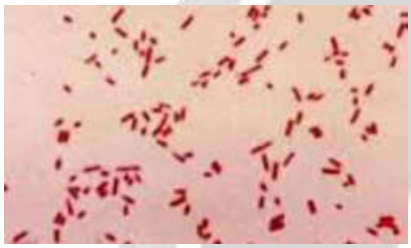


Fig2. Gram staining of *S. aureus*

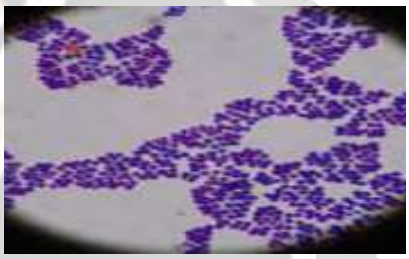
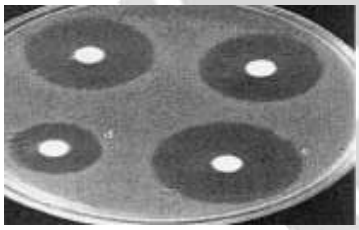


Fig 3 & 4. Showing the Antibacterial activity of *P.guajava E.coli S.aureus*



DISCUSSION

Investigating the antibacterial effect of guava leaves involved a comparison of its methanolic extract with commercially available antibiotics by comparing the inhibition zones. We have noted that the commercial antibiotics showed a larger inhibitory effect than the guava leaf methanolic extract. The demonstration of activity of the extract against both gram positive and gram negative is an indication of the broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these bacterial infections. **(Doughari JH *et al.*, 2008)**

In the previous investigation, some results were reported, in which *P. guajava* leaves were tested for their antibacterial activity using the agar diffusion technique, against bacteria such as *E. coli* and *S. aureus* by the paper disc diffusion method supported by the tubidimetric method.

The leaf of *P. guajava* has been widely used in the management of gastroenteritis and child diarrhea by the rural folk who cannot afford antibiotics. The leaf extracts of *P. guajava* inhibited the growth of bacteria in our study, in their study found Guava to be effective against *S. aureus*, and *E. coli*. Our study demonstrated the anti-microbial efficacy of the leaf extracts of *P. guajava* against bacteria and fungi. Guava also offers other health benefits as an excellent source of antioxidants and a good source of vitamin C. Guava leaf extract has activity against various groups of microorganism, such as *Escherichia coli* and *Staphylococcus aureus*.

P. guajava leaves have long been recognized for their antibacterial activity. They were shown to inhibit both Gram-positive and Gram-negative bacteria such as *Escherichia coli* and *Staphylococcus aureus*.

Conclusion

Due to the rapid emergence of drug-resistant pathogens natural products may occasionally be employed in place of antibiotics and chemotherapy drugs. Depending on the results the methanolic extract of P.guajava leaf may inhibit all of the microorganisms used in this investigation to varied degrees. The information gathered may provide confirmation for its purported medical applications. In conclusion the guava leaf methanolic extract is the most effective treatment for the tested bacterial strains of *Staphylococcus aureus* and *Escherichia coli.*

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